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NEW MICROMANIPULATOR AND METHODS FOR THE ISOLATION OF A SINGLE BACTERIUM AND THE MANIPULATION OF LIVING CELLS

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Barber's method for the isolation of bacteria by means of mechanically operated pipets has been used with considerable success, not only in the branch for which Barber primarily intended it, but also in experimental cytology and embryology. In 1912, the method was first applied to the dissection and injection of animal cells (Kite and Chambers¹), opening up a new field for investigation into the physical properties of protoplasm and the nature of cell structures.

The moist chamber devised by Barber and his method of making glass pipets and needles (Barber,² Chambers³), which are stiff and yet fine enough to puncture red blood corpuscles, leave nothing to be desired. Unfortunately, his instrument for manipulating the pipets, unless skilfully made, has too much lost motion, and wear and tear soon renders the movement jerky and undependable.

By using a new principle for moving the pipets, I have been able to construct an instrument which has the following advantages over Barber's: (a) simplicity of construction; (b) no lost motion through wear and tear; (c) accurate and continuous control of the movements of the needle or pipet tip in any direction under the highest magnification of the microscope; (d) maintenance of the needle tip in one focal plane while it is being moved back and forth in any of the three directions; and (e) existence of adjusting devices to facilitate placing the needle or pipet into position.

The basic principle of the instrument consists in rigid bars which are screwed apart against springs. The movements imparted are in arcs of a circle having a radius of about $2\frac{1}{2}$ inches. As the extreme range of movement of the fine adjustments is only 2 mm., the curvature of the arc is unnoticeable. The instrument is being patented.

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¹ Science, 1912, 36, p. 639.

² Philipp: Jour. Sc., Section B., Trop. Med., 1914, 9, p. 307.

³ Biol. Bull., 1918, 34, p. 121.

A MECHANICAL MICROMANIPULATOR FOR CONTROLLING THE MOVEMENTS OF A MICRONEEDLE OR MICROPIPET IN THE FIELD OF A COMPOUND MICROSCOPE

The principle of this device is demonstrated on considering the mechanism for the movements in one plane only (fig. 1). This consists of 3 bars of rigid metal connected at their ends to form a Z-like figure by resilient metal acting as a spring hinge. On turning certain screws the bars are forced apart; on reversing the screws the bars return to their original position, owing to the springs at the end of the bars. By these means arc movements may be imparted to the tip of a needle when placed in the proper position.

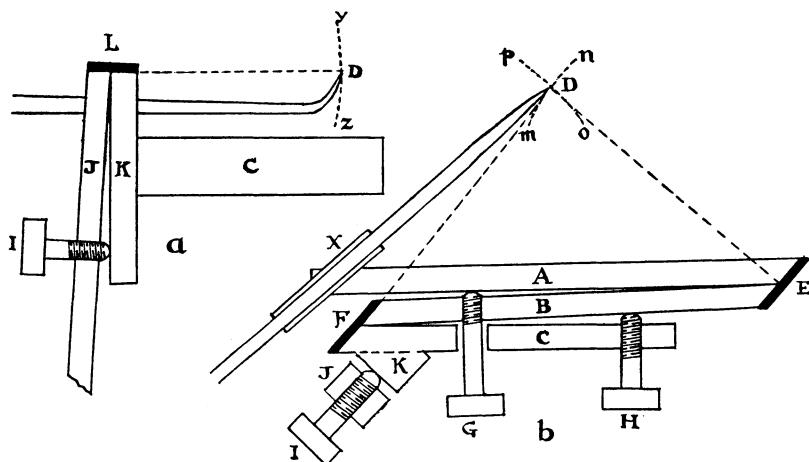


Fig. 1.—Diagram showing the working principle of the micromanipulator. In 1, a, where the instrument is viewed from the side, screw I moves the needle tip through the vertical arc y-z. In 1b, where the instrument is viewed from above, screws G and H move the needle tip through the horizontal arcs m-n and o-p.

The needle or any instrument, the tip of which is to be manipulated, is held in a carrier fastened to the free end of a bar, A, at x. The needle is made to extend so that its tip is at the apex of an imaginary triangle at D. In order to obtain 2 movements at right angles to one another and in the horizontal plane, the tip of the needle must be at the apex, D, of a right-angled isosceles triangle, the base of which is a straight line joining the centers, E and F, of the 2 springs holding the 3 bars, A, B, and C, together. The shank of screw G passes through a large hole in bar C, and is screwthreaded in bar B. Turning it spreads apart bars A and B and imparts an arc movement to the needle tip at D at right angles to that procured by turning screw H.

The movement in the vertical plane at right angles to the aforementioned movements is produced by screw, I (fig. 1, a), which is screw-threaded in a rigid vertical bar, J, and abuts against a vertical extension, K, of bar C. The extension, K, is parallel to the bar, J, and is connected to it at its top by means of a spring hinge. Turning screw, I, spreads apart bars, J and K, and lifts the whole combination (A, B, and C), and imparts an arc movement in the vertical plane to the tip of the needle at D. In order to procure a vertical movement the tip of the needle at D must lie in the same horizontal plane, L-D,

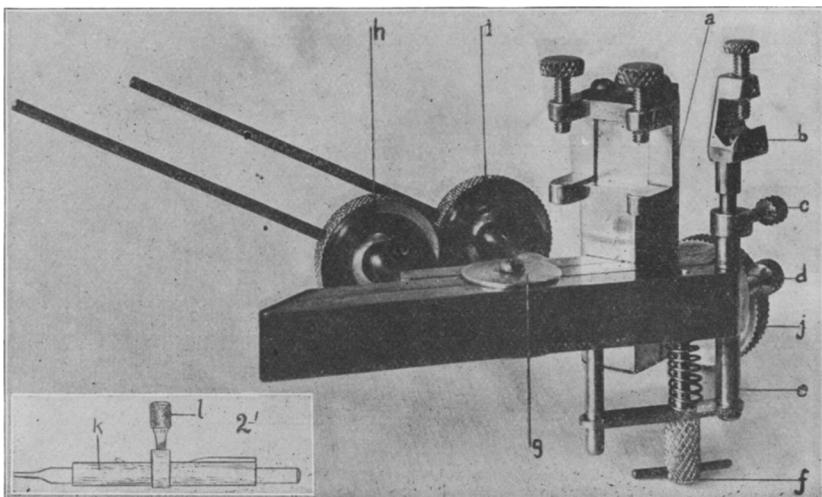


Fig. 2.—Left-handed micromanipulator to be clamped to microscope. a, stationary part with lugs by means of which instrument is clamped to microscope stage; b, holder for brass collar in which pipet is carried; c, screw to clamp post of holder at any desired height; d, screw to clamp main post of holder which revolves on its axis to produce a side-to-side coarse adjustment of pipet; f, coarse adjustment screw for raising and lowering; g, upper one of two disc guides for the horizontal bars; h, and i, fine adjustment screws for lateral movements; j, fine adjustment screw for vertical movement.

Fig. 2'.—Brass collar which is to be clamped at k into holder, b, of instrument to serve as coarse adjustment for the in-and-out movement of the pipet; l, screw which presses on a spring to clamp pipet in collar.

with the spring fastening K and J together. When screw, I, is turned, the needle tip will move in an arc, y to z, more nearly vertical than any other arc on the same circumference of which the point, D, is the center.

The micromanipulator may be furnished either with a clamping device (fig. 2, I) for fastening it directly to a square microscope stage (see fig. in article by Kahn⁴), or with a rigid pillar which rises from

⁴ Jour. Infect. Dis., 1922, 31, p. 344.

a large metal base on which the microscope is clamped. When fitted with a clamping device, the instrument depends for its steadiness on that of the microscope.⁵ The horizontal bars of the instrument extend diagonally across the corner below the level of the stage. They do not interfere with the substage accessories of the microscope nor with any of the known types of mechanical stages.⁶

The necessity of having one or two instruments is, of course, conditioned by the type of work to be done. For picking up bacteria one is sufficient. For microdissection in experimental embryology a great deal can be done with one instrument, but for cell injection in general and for tissue cell dissection two instruments are indispensable so that two needles or a needle and a pipet may be manipulated simultaneously.

When two instruments are to be used both must be placed at the front of the microscope so that the needles may extend, side by side, into the moist chamber from the front. As the horizontal bars of the instrument extend diagonally under the microscope stage, one must be a mirror image of the other. According to their position with respect to the microscope, they may be designated as right-handed and left-handed models. For bacteriologic work, in which it is more convenient to work from the left, the right-handed model is to be preferred, as it can be swung around and fastened to the left side so that the pipet may project into the moist chamber from the left.

THE SETTING UP AND THE WORKING OF THE INSTRUMENT

The instrument possesses devices to aid in the preliminary adjustment of the pipet or needle. One is the brass collar, fig. 2'. The pipet is first inserted into the collar where it is held in place by a spring. The collar is then clamped in the pipet holder of the instrument. This device facilitates sliding the pipet into or out of the moist chamber without danger of breaking or contaminating the tip of the pipet. For raising and lowering there are two adjusting devices. One is the telescoping pillar (fig. 2, c), for quickly adjusting the pipet to the height of the chamber; the second is operated by a spring screw (fig. 2, f) for bringing the pipet into focus. By these means the pipet tip is brought into the field of a low powered objective. Before centering the tip one must set the bars which control the fine adjustments into a state of tension by giving a few turns to the milled heads of each of

⁵ Steadiness may be assured by a brace, one end being screwed to the rigid vertical part of the instrument and the other end to the foot of the microscope.

⁶ In the case of the Bausch & Lomb and Spencer stages it may be necessary to replace the screw clamping the front end of the stage for one with a shorter head.

the three screws. The needle tip is then more or less accurately centered and finally raised close to the hanging drop by means of the preliminary adjustments. The instrument is now ready for action.

The milled heads of the screws which control the lateral movements (fig. 2, h and i) may be provided with levers to increase the delicacy of their manipulation. The screw, j, controlling the vertical movement may be furnished with a wire wound flexible shaft about 18 inches long. Curving the shaft around one side of the microscope brings the control of this screw, which is the one most frequently used, close to that of the fine adjustment of the microscope. The shaft also facilitates the use of both hands for the various movements of the one instrument (see fig. in Kahn's article).

The micromanipulator is intended to be used with the mechanical stage of the microscope. The mechanical stage moves the moist chamber. As the cell or tissue to be manipulated lies in a drop hanging from the roof of the chamber, the motion imparted by the mechanical stage moves the cells against the microneedle. Indeed, most of the dissection, when a single needle is used, is done by first bringing the needle tip into the cell and then dragging the cell away by means of the mechanical stage.

The horizontal movements of the micromanipulator are used mostly for the purpose of bringing the tip of the needle accurately into a desired spot in the field of the microscope preparatory to the actual operative work. In order to insure the greatest possible steadiness to the vertical movement, the part of the instrument which produces this movement adjoins, and is manipulated from, the stationary and rigid part of the instrument. To make this possible the present design incorporates a theoretical error which can be understood from fig. 1. Turning screw I to produce the vertical movement throws the combination of bars A, B, and C out of the horizontal, and it is these bars on which the lateral movements of the needle depend. However, the angle at which these bars are placed minimizes the error so as to be practically unnoticeable.

Guides exist in the instrument to insure a true travel of the bars as they spread apart or come together. The guide for the bar which produces the vertical movement consists of a depression in the stationary part of the instrument into which the vertical bar fits. The guides of the lateral movements are two metal disks which can be tightened or loosened by screws. The upper one is seen in fig. 2, f.

They correct two possible errors which may occur on reversing the direction of movement, namely, a dropping of the needle or pipet out of focus and a shifting to one side.

The first error can be corrected by tightening one or both of the guides; the second, by loosening them. The guides, therefore, must be neither too tight nor too loose. The first error is the more serious of the two. It is due to an unequal tension in the springs which throws the tip of the moving screw to a different spot on the bar against which it abuts. If this be not corrected, the screw will, in time, wear a depression in the brass bar that is out of center, thus perpetuating the error. The second error is due to the fact that the guides are too tight so that they bind and prevent the bars from making a true return. If not corrected, this error will gradually be eliminated with the wear of the frictional surfaces.

By an accidental knock the horizontal bars of the instrument may be jarred out of place or the fine adjustment screws injured. If the upper and lower surfaces of the horizontal bars are not flush, loosen the guide disks (fig. 2, f) also the screws of the springs on the ends of the bars and, with a wooden mallet, gently hammer the bars until they are flush. Then tighten the guide disks to keep the bars flush and carefully tighten the screws of the springs. If the screws have been bent by the accident they must be changed, otherwise tightening them will again pull the bars out of place. If the guide disks are bent, they also must be changed. A more serious accident occurs when the fine adjustment screws are injured. The steel shafts of the screws may be bent or they may have cut into the brass so as to loosen the threads. This tends to throw the shaft of the screw out of center. In such a case a new screw must be procured and accurately centered opposite the bar against which it abuts.

THE SUBSTAGE CONDENSOR AND THE METHOD OF MAKING BARBER'S MOIST CHAMBER AND GLASS NEEDLES

For critical illumination the height of the moist chamber must be equal to the working focal distance of the substage condensor. The Abbe condensor can be used by removing the top lens. The focal distance of the remaining lens is almost one inch. In the Bausch and Lomb microscope the substage can easily be arranged to raise this lens sufficiently to have at least half its focal distance above the surface of the stage. This is ample if one is satisfied with a moist chamber no

higher than half an inch. The focal distance of this lens can be reduced and its illuminating power correspondingly increased by placing the lens of a 10 X dissecting lens on top of it. This combination has a focal distance of about $\frac{3}{8}$ of an inch and, if the substage can be raised to bring the top lens flush with the upper surface of the stage, all of this distance may be used for the height of the moist chamber. Better results are secured with a triple lens condenser with its top lens removed. Such a condenser from Leitz, which I am using, has a working focal distance of just $\frac{3}{8}$ of an inch. One may also use condensers which are made with a specially long working distance for projection apparatus in which a cooling trough is placed between the condenser and the slide.

If the working focal distance of the condenser is less than $\frac{3}{8}$ of an inch, it is well to have two moist chambers, one for critical work and the other, from $\frac{3}{8}$ to $\frac{1}{2}$ inch high, for ordinary work. This is advisable because it is easier to make needles for the higher chamber.

The moist chamber is made of glass. There is convenient form for cytological purposes with the open end designed to face the front of the microscope. The base is a fairly thin glass slide about $2\frac{3}{8} \times 2$ inches in size. The sides consist of strips of plate glass about $1\frac{7}{8}$ inches long and $\frac{1}{4}$ inch wide and of a height determined by the available condenser. One end of the chamber is closed with a strip of glass of the same height as the sides and backed by another strip a fraction higher in order to prevent a coverslip from sliding beyond it. The trough of the chamber should be from $\frac{3}{4}$ to $\frac{7}{8}$ of an inch wide. The strips are cemented with any ordinary glass cement. Heated Canada balsam serves well. Care must be taken to have the upper surfaces of the strips horizontal. This may be done while the cement is still soft by focusing on the upper surface of the strips and by manipulating the strips until all parts of their surfaces lie in one focal plane.

To maintain moisture in the chamber strips of wet blotting paper may be placed across its inner end and along its sides.

This moist chamber is designed for coverslips 24×40 mm. The coverslip is sealed on the chamber with petrolatum. Square or round coverslips may also be used provided the rest of the chamber be roofed with other strips of coverglass.

The hanging drop containing the cells or tissue to be operated on is placed on the coverslip, which is then inverted over the moist chamber.

To prevent the petrolatum from spreading on the coverglass and from contaminating the hanging drop a thin film of melted paraffin may be spread and cooled on the coverglass bounding the area to be occupied by the hanging drop.

The moist chamber is open at one end to permit the entrance of the microneedles or pipets. To prevent undue evaporation, especially when a preparation is to be left over night, the open end may be temporarily closed by means of a paraffined, thin cardboard trough. The trough is placed over the shanks of the needles and filled with soft petrolatum containing a few threads of cotton to give substance to the petrolatum. The petrolatum closes around shafts of the needle and seals the opening of the chamber without interfering with the movement of the needles. To prevent the petrolatum from spreading on the floor of the moist chamber it is well to have a shallow pan of cardboard set under the shanks of the needles for the trough to rest on.

The needles are made either from soft or hard glass tubing. When a brass collar is used (fig. 2) the glass tubing should be selected to fit it. The collars furnished with the instrument receive tubing about $\frac{1}{8}$ inch in outside diameter. As regards the wall of the glass tubing in general, it seems to be true that the thicker the wall the firmer tends to be the tip of the needle. The method of making the needle is given in a paper of Barber's² and in one of mine.³ A brief account will suffice here. Acetylene or ordinary illuminating gas may be used. For a microburner use a piece of hard glass tubing bent at right angles and with the burner end closed except for the smallest aperture that will retain a flame. This may be done by heating the end and pinching it with forceps. The size of the flame may be regulated by a screw pinch cock on the rubber tube.

To make the needles proceed as follows: 1. In an ordinary burner draw out one end of a glass tube with a capillary of about 0.3-0.5 mm. in diameter. 2. Lower the flame of the microburner to the smallest flame possible. Hold the shank of the tube in the left hand and grasp the capillary at its end either with the thumb and finger of the right hand or with forceps having flat tips coated with Canada balsam. Bring the capillary over the flame and pull gently until the capillary parts. The hands should remain on the table during the process and, as the capillary parts, lift the glass away from the flame by turning the hands slightly outward. The capillary will separate with a slight tug. The tip should be like that in c. If too little heat is used

and the pull made too suddenly, the capillary will part with a snap and have a broken tip. If too much heat is used the tip will be drawn out into a long hair, e. 3. Bend the capillary at right angles by heating it just back of the point and pushing up with a dissecting needle, b. The length of the needle beyond the bend is conditioned by the height of the moist chamber to be used. The type of needle shown in g is used for cutting by bringing the upper limb of the needle below and up into the cell. 4. The micropipets are made from needles of the kind shown in d. The needle is first inserted in the brass collar (fig. 2') and connected with a rubber tube to be blown into by mouth or with my micro-injection apparatus (cf. Chambers⁷). It is then placed in the carrier of the instrument and the tip brought up into a droplet suspended in the moist chamber. By jamming the tip against the cover-glass the hair tip breaks off converting the needle into a pipet. During this procedure blow into the hollow needle to prevent clogging of the pipet by glass fragments which tend to be drawn in by capillarity.

OTHER APPARATUS HITHERTO USED FOR MICRO-OPERATIVE WORK

Barber's instrument is based on the principle of a carrier pushed along a groove by a screw at one end. By having a series of three carriers built up on one another, each traveling in a different direction, movements in any one of three dimensions may be imparted to a needle clamped to the top carrier. Hecker⁸ improved Barber's instrument, but added materially to the intricacy of its make-up.

Other investigators that I know of who have devised instruments for micro-operative work are Schmidt,⁹ Chabry,¹⁰ Schouten,¹¹ Tchahotine,¹² McClendon,¹³ Malone,¹⁴ Bishop and Tharaldsen.¹⁵

Schmidt's instrument is one of historic interest only. I have already described it.³ Chabry used a delicate spring device with which he could shoot the tip of a glass needle into an ovum to any desired depth. Schouten uses his for the isolation of bacteria. It consists of a pillar carrying a needle which may be mechanically raised or lowered. For

⁷ Anat. Rec., 1922, 24 p. 1.

⁸ Jour. Infect. Dis., 1916, 19, p. 306.

⁹ New Orleans Med. Jour., 1869, 22, p. 627; 1870, 23, pp. 66 and 274.

¹⁰ Jour. de l'Inst. et de Physiol., 1887, 25, p. 167.

¹¹ Ztschr. wiss. Mikrosc., 1905, 22, p. 10; Königl. Akad. Wetensch. Amsterd., 1911, 13, p. 840.

¹² Ztschr. wiss. Mikrosc., 1912, 29, p. 188; Biol. Centralbl., 1921, 32, p. 623.

¹³ Biol. Bull., 1907, 12, p. 141.

¹⁴ Jour. Path. & Bacteriol., 1918, 22, p. 222.

¹⁵ Amer. Nat., 1921, 55, p. 381.

the horizontal movements, Schouten depends on pushing the microscope on a flat base. McClendon attached an up and down movement to a Spencer mechanical stage. Tchahotine used a mechanism attached to the tube of his microscope from which extended a glass needle curved in such a way as to bring its tip into the microscopic field where it was brought into focus. Tissues are dissected under a low power objective by moving the microscope tube and by pushing the cells against the needle tip by means of the mechanical stage of the microscope. Malone uses Schouten's method, but, instead of having a special pillar with a raising device, he mounts his pipet carrier on the tube of a second microscope whose adjustments serve as a means for raising and lowering the pipet. Bishop and Tharaldsen have a simple instrument based on a principle somewhat resembling mine but lacking in proper control for one of the two lateral movements. Recently I have heard that Zeiss is manufacturing a microdissection instrument which, however, is said to be of an intricate design.